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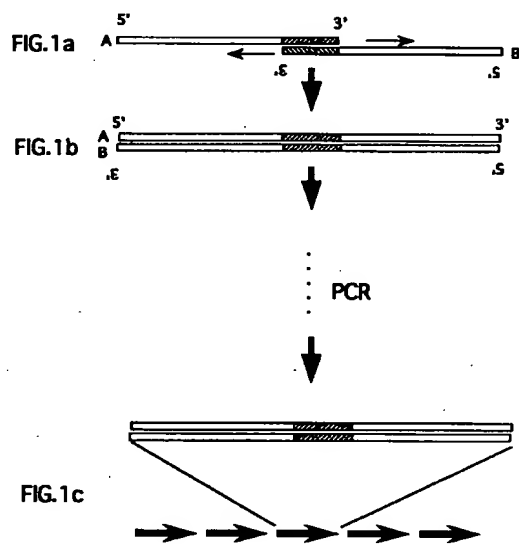
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(54) **A method of forming a macromolecular microgene polymer**

(57) A method of forming a macromolecular microgene polymer comprises allowing DNA polymerase to act on oligonucleotides A and B complementary at least partially to each other to effect polymerase chain reaction. According to the present invention, there can be obtained a polymer consisting of a repeating microgene, which is efficiently and simply formed.



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Description

FIELD OF THE INVENTION

5 The present invention relates to a method of forming a macromolecular microgene polymer by use of DNA polymerase.

BACKGROUND OF THE INVENTION

10 The advent of evolutionary molecular engineering has made it feasible to create an enzyme (protein) forming the basis of life reaction or a gene coding therefor in laboratories. By this technology, an enzyme (protein) with new activity not occurring in nature can be produced and expected for use in various applications to the fields of medicine and engineering.

15 An enzyme (protein) or a gene coding therefor is composed of a polymer of amino acids or nucleotides as a block unit. In evolutionary molecular engineering, a molecule with desired activity is selected from a pool of polymers consisting of random amino acid or nucleotide block units.

20 However, even if it is attempted to prepare polymers with every combination, there is a limit to the physical amount of compounds which can be synthesized, so there is a limit to the number of blocks which can be linked, and as a consequence, a too large protein or gene cannot be created. Further, in consideration of an *in vitro* evolutionary system for translating a protein from a nucleic acid polymer, the appearance of "termination codon" terminating the translation is a great problem. Therefore, a microgene which is large to a certain extent is preferably used as a block unit to form a gene coding for a large protein.

25 There is the hypothesis that a large gene was born by repeatedly polymerizing a small gene (microgene) (S. Ohno & J. T. Epplen, Proc. Natl. Acad. Sci. U.S.A. 80:3391-3395). Because it is considered that a polypeptide rich in a simple repeating structure can easily have a stable secondary structure, evolutionary molecular engineering directed at large proteins or genes requires the techniques of repeatedly polymerizing a short structural unit to synthesize a macromolecule (Nature 367:323-324, 1994).

30 At present, a rolling circle synthesis method is reported as a method of preparing a polymer consisting of a short repeating DNA unit (PNAS 92:4641-4645, 1995).

35 However, this method should go through a plurality of steps including phosphorylation reaction, linkage reaction, polymerization reaction, double-stranded chain forming reaction, so its complicated reaction system is problematic.

Under these circumstances, there have been demands for developments in a reaction system in which a gene polymer can be formed more simply.

SUMMARY OF THE INVENTION

The object of the present invention is to provide a method of efficiently and simply forming a polymer consisting of a repeating microgene.

40 As a result of their extensive research, the present inventors found that a macromolecular microgene polymer can be formed efficiently and simply by allowing DNA polymerase to act on oligonucleotides complementary at least partially to each other, to complete the present invention.

That is, the present invention is a method of forming a macromolecular microgene polymer, which comprises allowing DNA polymerase to act on oligonucleotides A and B complementary at least partially to each other to effect polymerase chain reaction (PCR).

45 The DNA polymerase includes exonucleases, particularly those acting in the 3'→5' direction. In addition, the DNA polymerase is preferably thermally stable.

In the method of forming a macromolecular microgene polymer according to the present invention, the 3-terminals of oligonucleotide A and/or oligonucleotide B can contain at least one nucleotide not capable of forming a base pair with the other oligonucleotide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 a to FIG. 1 c is a schematic drawing showing the method of the present invention.

FIG. 2 is a photograph showing the result of agarose gel electrophoresis.

55 FIG. 3 shows genes synthesized by the method of the present invention.

FIG. 4 is a photograph showing the result of agarose gel electrophoresis.

FIG. 5 is a photograph showing the result of agarose gel electrophoresis.

FIG. 6 is a photograph showing the result of agarose gel electrophoresis.

FIG. 7 is a photograph showing the result of agarose gel electrophoresis.

FIG. 8 is a photograph showing the result of agarose gel electrophoresis.

FIG. 9 shows genes synthesized by the method of the present invention.

FIG. 10 is a photograph showing the result of SDS polyacrylamide gel electrophoresis.

5 DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention is described in detail.

As shown in FIG. 1a, two oligonucleotides (oligonucleotides A and B) with complementary regions being at least partially to each other are synthesized before conducting polymerase chain reaction according to the present invention.

10 In the present invention, oligonucleotides A and B are synthesized so as to be complementary to each other particularly in their 3'-terminal sequences. The number of oligonucleotides forming a complementary chain to each other is preferably at least 6, more preferably at least 8, although there is no particular limitation.

Alternatively, oligonucleotides A and B may be synthesized such that the 3-terminals of oligonucleotide A and/or oligonucleotide B contain 1 or more nucleotides (preferably 1 to 3 nucleotides) being not capable of forming base pairs 15 with the other oligonucleotide. By this operation, the efficiency of reaction can be raised.

Further, because one object of the present invention is to create a completely new gene polymer not occurring in the nature, said 2 oligonucleotides are not particularly limited and may be selected arbitrarily insofar as they are at least partially complementary to each other. The synthesized oligonucleotides form a double-stranded chain in only the part of their complementary region.

20 As used herein, the term "complementary" can refer not only to the relationship between adenine and thymidine or guanine and cytosine, but also to the relationship between guanine and thymine or the like insofar as oligonucleotides A and B are at least partially complementary to each other.

Oligonucleotides A and B function as primers to initiate PCR at their complementary region (a double-stranded chain in FIG. 1a), where the single-stranded chain (i.e. not forming the double-stranded chain with oligonucleotide B) of oligonucleotide A acts as a template for synthesizing oligonucleotide B and the single-stranded chain (i.e. not forming 25 the double-stranded chain with oligonucleotide A) of oligonucleotide B acts as a template for synthesizing oligonucleotide A (FIG. 1a). If PCR is conducted by allowing e.g. thermostable DNA polymerase with the 3'→5' exonuclease activity to act on said 2 oligonucleotides, double-stranded DNA is synthesized as a repeating unit (FIG. 1b). By further continuing the PCR, large DNA consisting of continuous repeating units is synthesized (FIG. 1c).

30 The PCR using polymerase (e.g. Taq polymerase) is carried out by conducting 1 cycle at 94 °C for 10 to 120 seconds, 30 to 65 cycles each at 69°C for 10 to 120 seconds, and 1 cycle at 69 °C for 3 to 7 minutes.

To conduct PCR efficiently, additional reaction at 94 °C for 10 minutes and at 69 °C for 10 minutes is preferably carried out before conducting the above cycles.

35 In this manner, the complementary part of the 2 oligonucleotides serves as a self-primer and the other oligonucleotide serves as a template for synthesizing them, resulting in polymerization of DNA with double-stranded chain DNA as a repeating unit (FIG. 1b) with an extremely large number of copies in the same direction (FIG. 1c). In the present invention, the replacement, insertion and/or deletion of several nucleotides may occur between repeating units insofar as the repeating units form a complementary chain.

40 EXAMPLES

Hereinafter, the present invention is described in more detail by reference to Examples which however are not intended to limit the scope of the present invention.

45 Example 1

KY-794 (SEQ ID NO:1) and KY-795 (SEQ ID NO:2) were synthesized respectively as oligonucleotides A and B for use in PCR. The synthesized oligonucleotides A and B were composed of 22 and 23 nucleotides respectively where their 3'-terminal 8 nucleotides were complementary to each other (the sequence at the 15- to 22-positions in KY-794 50 was complementary to the sequence at the 15- to 22-positions in KY-795). Adenine (A) was added to the 3'-terminal of KY-795 to prevent formation of a base pair with KY-794.

The conditions for PCR using the above oligonucleotides in a 50 µl reaction volume are as follows:

KY-794 (SEQ ID NO:1)	20 pmol
KY-795 (SEQ ID NO:2)	20 pmol
dNTP	350 μ M
MgCl ₂	1.75 mM
Tris-HCl, pH 9.2	50 mM
(NH ₄) ₂ SO ₄	14 mM
Taq polymerase	2.6 units/50 μ l

The Taq polymerase used was a mixture of Taq polymerase and Pwo polymerase contained in Expand™ Long Template PCR system (Boehringer).

PCR was carried out using 9600 or 2400 PCR system (Perkin Elmer) for cycle reaction under the following conditions:

94°C 10 minutes

69°C 10 minutes

(94 °C for 10 seconds and 69°C for 60 seconds) \times 45 cycles

69°C 7 minutes

The enzyme was added when the system reached 94 °C.

The PCR product obtained under these conditions was subjected to 1.2 % agarose gel electrophoresis.

The result is shown in FIG. 2.

As can be seen from FIG. 2, DNA reaching several kilo base pairs or more can be polymerized in this method.

The polymer thus obtained was cloned into plasmid vector pTZ19R (Mead et al., Protein Eng. 1:67-74 (1986)). For 4 clones (pSA32, pSA33, pYT5 and pYT8), their insert fragments were sequenced using a sequencer (Perkin Elmer).

The results are shown in FIG. 3. The nucleotide sequences determined for the respective clones are shown in SEQ ID NO: 3 for pSA32, SEQ ID NO: 4 for pSA33, SEQ ID NO: 5 for pYT5, and SEQ ID NO: 6 for pYT8.

In SEQ ID NO:3, the sequences at the 1- to 36-positions, the 40- to 75-positions and the 77- to 112-positions are identical with one another, so it is understood that a polymer was synthesized in which many of double-stranded chains as repeating units each consisting of 37 base pairs derived from KY-794 and KY-795 had been linked in the same direction. This applies to SEQ ID NOS:4-6.

In FIG. 3, "Δ" indicates the absence of the corresponding nucleotide in the linking region of the repeating units each consisting of the sequence derived from the oligonucleotides, and the underlined nucleotides are an insert of unknown origin in the linking region of the repeating units.

Example 2

In the reaction shown in Example 1 (FIG. 2), the 3'-terminal of KY795 had one nucleotide being not capable of forming a base pair with KY-794. In this example, polymerization was carried out using the combination of oligonucleotide KY-783 (SEQ ID NO:7) and oligonucleotide KY-794, i.e. the combination not forming such a mismatch.

The conditions for PCR were identical to those in Example 1. The PCR product obtained under these conditions was subjected to 1.2 % agarose gel electrophoresis.

The result is shown in FIG. 4.

As can be seen from FIG. 4, the efficiency of polymerization is improved when at least one nucleotide being not capable of forming a base pair with the other oligonucleotide is present at the 3'-terminal of the oligonucleotide (FIG. 4, lane 2).

Example 3

As shown in FIG. 5, KY-794 and KY-795 have a complementary region of 8 bases. In this example, polymerization

was carried out using oligonucleotide KY-845 (SEQ ID NO:8) and oligonucleotide KY-846 (SEQ ID NO:9) whose complementary region consisted of 6 nucleotides which is shorter by 2 bases than above. The composition of the reaction solution was the same as in Example 2 except that PCR was carried out under the following cycle conditions 1 or 2:

5 (Conditions 1)

94°C 10 minutes
63°C 10 minutes

10 (94 °C for 10 seconds and 63°C for 60 seconds) × 45 cycles

63°C 7 minutes;

(Conditions 2)

15

94°C 10 minutes
66°C 10 minutes

(94 °C for 10 seconds and 66°C for 60 seconds) × 45 cycles

20

66°C 7 minutes

The PCR products obtained under these conditions were subjected to 1.2 % agarose electrophoresis.

The results are shown in FIG. 5.

25

In FIG. 5, lanes 2 and 3 were obtained under Conditions 1 and lanes 4 and 5 under Conditions 2.

As can be seen from lanes 1 and 2 in FIG. 5, the polymerization reaction proceeds by decreasing the annealing temperature of the PCR cycle to 63°C even by the combination of the oligonucleotides having a complementary region of as short as 6 bases.

30 Example 4

In this example, thermostable DNA polymerase having the 3'→5' exonuclease activity was used as an enzyme for PCR. The 3'→5' exonuclease activity is important for raising polymerization efficiency. Accordingly, the importance of the 3'→5' exonuclease activity was examined using thermostable DNA polymerase lacking in the 3'→5' exonuclease activity.

35

The oligonucleotides used were KY-794 (SEQ ID NO:1) and KY-785 (SEQ ID NO:2). The PCR reaction solution had the same composition as in Example 1 except that the enzyme was 1.9 units/50 µl of thermostable polymerase Pfu DNA polymerase commercially available from Stratagene or Exo-Pfu DNA polymerase assumed to lack the 3'→5' exonuclease activity. PCR was carried out under the same cycleconditions as in Example 1. The PCR product obtained

40

under these conditions was subjected to 2 % agarose gel electrophoresis.

The result is shown in FIG. 6.

As can be seen from FIG. 6, polymerization efficiency was dropped where Exo-Pfu DNA polymerase assumed to lack the 3'→5' exonuclease activity was used(lane 3) as compared with the case where Pfu DNA polymerase having the 3'→5'exonuclease activity was used (lane 2).

45

Example 5

In this example, polymerization was carried out using oligonucleotides with various sequences.

As shown in FIG. 7, the combination of KY-794 (SEQ ID NO:1) and KY-795 (SEQ ID NO:2) and the combination of KY-808 (SEQ ID NO:10) and KY-809 (SEQ ID NO:11) are identical in the number (= 8) of nucleotides forming a complementary chain, but are greatly different in the nucleotide composition of the complementary region.

50

KY-827 (SEQ ID NO:12), KY-828 (SEQ ID NO:13), KY-829 (SEQ ID NO:14) and KY-830 (SEQ ID NO:15) are partially modified sequences of KY-794 (SEQ ID NO:1), and KY-831 (SEQ ID NO:16), KY-832 (SEQ ID NO:17), KY-833 (SEQ ID NO:18), KY-834 (SEQ ID NO:19) and KY-835 (SEQ ID NO:20) are partially modified sequences of KY-795 (SEQ ID NO:2).

55

PCR was carried out under the same conditions as in Example 1 by using each of the following combinations: KY-794 (SEQ ID NO:1) and KY-795 (SEQ ID NO:2); KY-808 (SEQ ID NO:10) and KY-809 (SEQ ID NO:11); KY-827 (SEQ ID NO:12) and KY-795 (SEQ ID NO:2); KY-828 (SEQ ID NO:13) and KY-795 (SEQ ID NO:2); KY-829 (SEQ ID NO:14) and KY-795 (SEQ ID NO:2); KY-830 (SEQ ID NO:15) and KY-795 (SEQ ID NO:2); KY-794 (SEQ ID NO:1) and KY-831

(SEQ ID NO:16); KY-794 (SEQ ID NO:1) and KY-832 (SEQ ID NO:17); KY-794 (SEQ ID NO:1) and KY-833 (SEQ ID NO:18); KY-794 (SEQ ID NO:1) and KY-834 (SEQ ID NO:19); and KY-794 (SEQ ID NO:1) and KY-835 (SEQ ID NO:20). The PCR products obtained under these conditions were subjected to 2 % agarose gel electrophoresis.

The results are shown in FIGS. 7 and 8.

As can be seen from FIGS. 7 and 8, there are differences in efficiency but the polymerization reaction proceeds in any of the combinations of oligonucleotide sequences used.

Example 6

In order to allow the sequence of the resulting polymer to have diversity, polymerization was carried out using a partially randomized oligonucleotide. KY-812 (SEQ ID NO:21) and KY-795 (SEQ ID NO:2) were used as primers. KY-812 (SEQ ID NO:21) is an oligonucleotide synthesized such that A, T, G or C is located at the 3- and 11-positions. The PCR reaction was carried out in the same manner as in Example 1. After the reaction, the resulting polymer was cloned into plasmid vector pTZ19R. For 4 clones (pYT15, pYT16, pYT20 and pYT21), their insert fragments were sequenced.

The results are shown in Table 9. The nucleotide sequences determined for the respective clones are shown in SEQ ID NO:22 for pYT15, SEQ ID NO:23 for pYT16, SEQ ID NO:24 for pYT20 and SEQ ID NO:25 for pYT21.

As can be seen from FIG. 9, the base at the 3-position had a preference for C, while A, T, G or C appeared as the base at the 11-position, so diversity was given to the sequence of the polymer.

Example 7

The protein encoded by the resulting polymer can be expressed in *E. coli*. The polymer obtained by the combination of KY-794 (SEQ ID NO:1) and KY-795 (SEQ ID NO:2) and the polymer obtained by the combination of KY-812 (SEQ ID NO:21) and KY-795 (SEQ ID NO:2) were cloned respectively into expression vector pET23b to give recombinants pYT32 and pYT33. The proteins derived from the polymers encoded by pYT32 and pYT33 were expressed in *E. coli* BL21 (DE3) and their cell extract was analyzed by SDS polyacrylamide gel electrophoresis on 15-25 % gradient gel.

The results are shown in FIG. 10. The molecular markers are of 97,400, 66,267, 42,400, 30,000, 20,100 and 14,000.

As can be seen from FIG. 10, proteins with a molecular weight of about 16 kDa derived from the polymers are expressed.

As illustrated above, a polymer consisting of a repeating microgene can be formed efficiently and simply according to the present invention.

SEQUENCE LISTING

5

SEQ ID NO: 1

LENGTH: 22

10

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

15

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACC TGCACAAAGG CG

22

20

SEQ ID NO: 2

LENGTH: 23

25

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

30

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGGGATCCAC TGCACGCCCTT TGA

23

35

SEQ ID NO: 3

LENGTH: 185

40

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

45

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCCG

50

GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCCGCGG

55

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCGCCCC

GACGGTCACCTGCACAAAGGCGTGCACTGGATCC

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCG

185

SEQ ID NO: 4

LENGTH: 162

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCGCG

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCGT

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCGCCA

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCGCCA

GACGGTCAC

162

SEQ ID NO: 5

LENGTH: 280

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCGCCG

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCGT

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCG

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCGCCG

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCC

GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCC
 GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCCGCCGG
 GACGGTCACCTGCACAA

280

SEQ ID NO: 6

LENGTH: 246

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACCTGCACAAAGGCGTGCA GTAGATCCCGCCCCG
 GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCCGC
 GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCCGCCGG
 GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCC
 GACGGTCACCTGCACAAAGGCGTGCA GTGGATCCCG
 GACGGTCACCTGCACAAAGGCG

646

SEQ ID NO: 7

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGGGATCCAC TGCACGCCTT TG

22

SEQ ID NO: 8

LENGTH: 20

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACC TGCACAGGCG

20

SEQ ID NO: 9

LENGTH: 21

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGGGATCCAC TGCACGCCTG A

21

SEQ ID NO: 10

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGACACC TGCAAACGGA GC

22

SEQ ID NO: 11

LENGTH: 23

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGGGATCCAC TGCAGCTCCG TTA

23

SEQ ID NO: 12

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CTGGGTCACC TGCACAAAGG CG

22

SEQ ID NO: 13

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACCCACACC TGCACAAAGG CG

22

SEQ ID NO: 14

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTGTGC TGCACAAAGG CG

22

SEQ ID NO: 15

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACG ACCACAAAGG CG

22

SEQ ID NO: 16

LENGTH: 23

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGGGATCCAC TCGTCGCCTT TGA

23

SEQ ID NO: 17

LENGTH: 23

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGGGATCCTG AGCACGCCTT TGA

23

5

SEQ ID NO: 18

LENGTH: 23

10

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

15

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGGGAAGGAC TGCACGCCTT TGA

23

20

SEQ ID NO: 19

LENGTH: 23

25

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

30

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CGCCTTCCAC TGCACGCCTT TGA

23

35

SEQ ID NO: 20

LENGTH: 23

40

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

45

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GCGGATCCAC TGCACGCCTT TGA

23

50

55

SEQ ID NO: 21

LENGTH: 22

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GANGGTCACC NGCACAAAGG CG

22

SEQ ID NO: 22

LENGTH: 314

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCGCCCGGCACAAAGGCGTGCACTGGATCCCG

GACGGTCACCCGACACAAAGGCGTGCACTGGATCCCG

GACGGTCACCCGACACAAAGGCGTGCACTGGATCCCG

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCCG

GATGGTCACCAGCACAAAGGCGTGCACTGGATCCC

GACGGTCACCCGACACAAAGGCGTGCACTGGATCCCG

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCC

GACGGTCACCAGCACAAAGGCGTGCACTGGATCCCG

GACGGTCACCTGCACAAAGGCGTGCACTGGATCCC

314

SEQ ID NO: 23

LENGTH: 408

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG

GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCC

GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG

GATGGTCACCGGCACAAAGGCGTGCAGTGGATCCC

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGT

GAGGGTCACCTGCACAAAGGCGTGCAGTGGATCCC

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCC

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCGG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG

GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG

GACGGTCAC

408

SEQ ID NO: 24

LENGTH: 674

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

GGTCACCGGCACAAAGGCGTGCAGTGGATCCCGCCGG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCGG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCGG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG

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10
15
20
25
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCC
GACGGTCACCAGCACAAAGGCGTGCAGTGGATCCC
GAAGGTCACCCGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCC
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCC
GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCGCCGG
GAAGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
GAAGGTCACCGGCACAAAGGCGTGCAGTGGATCCC
GACGGTCACCAGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCC
GACGGTCACCCGCACAAAGGCGTGCAGTGGATCCCGCCGG
GATGGTCACCGGCAC

674

30
SEQ ID NO: 25

LENGTH: 373

TYPE: nucleic acid

35
STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

40
SEQUENCE:

45
50
55
GAGGGTCACCCGCACAAAGGCGTGCAGTGGATCCCGCCGG
GACGGTCACCTGCACAAAGGCGTGCATTGGATCCCGCCGG
GACGGTCACCGGCACAAAGGGGTGCAGTGGATCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCGG
GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCC
GATGGTCACCCGCACAAAGGCGTGCAGTGGATCCC
GATGGTCACCCGCACAAAGGCGTGCAGTGGATCCCGCCGG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCC

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG

GAAGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG

373

Claims

1. A method of forming a macromolecular microgene polymer, which comprises allowing DNA polymerase to act on oligonucleotides A and B complementary at least partially to each other to effect polymerase chain reaction.
2. The method of forming a macromolecular microgene polymer according to claim 1, wherein the DNA polymerase contains an exonuclease acting in the 3'→5' direction.
3. The method of forming a macromolecular microgene polymer according to claim 1, wherein the DNA polymerase is thermally stable.
4. The method of forming a macromolecular microgene polymer according to claim 1, wherein the 3-terminals of oligonucleotide A and/or oligonucleotide B contain at least one nucleotide not capable of forming a base pair with the other oligonucleotide.

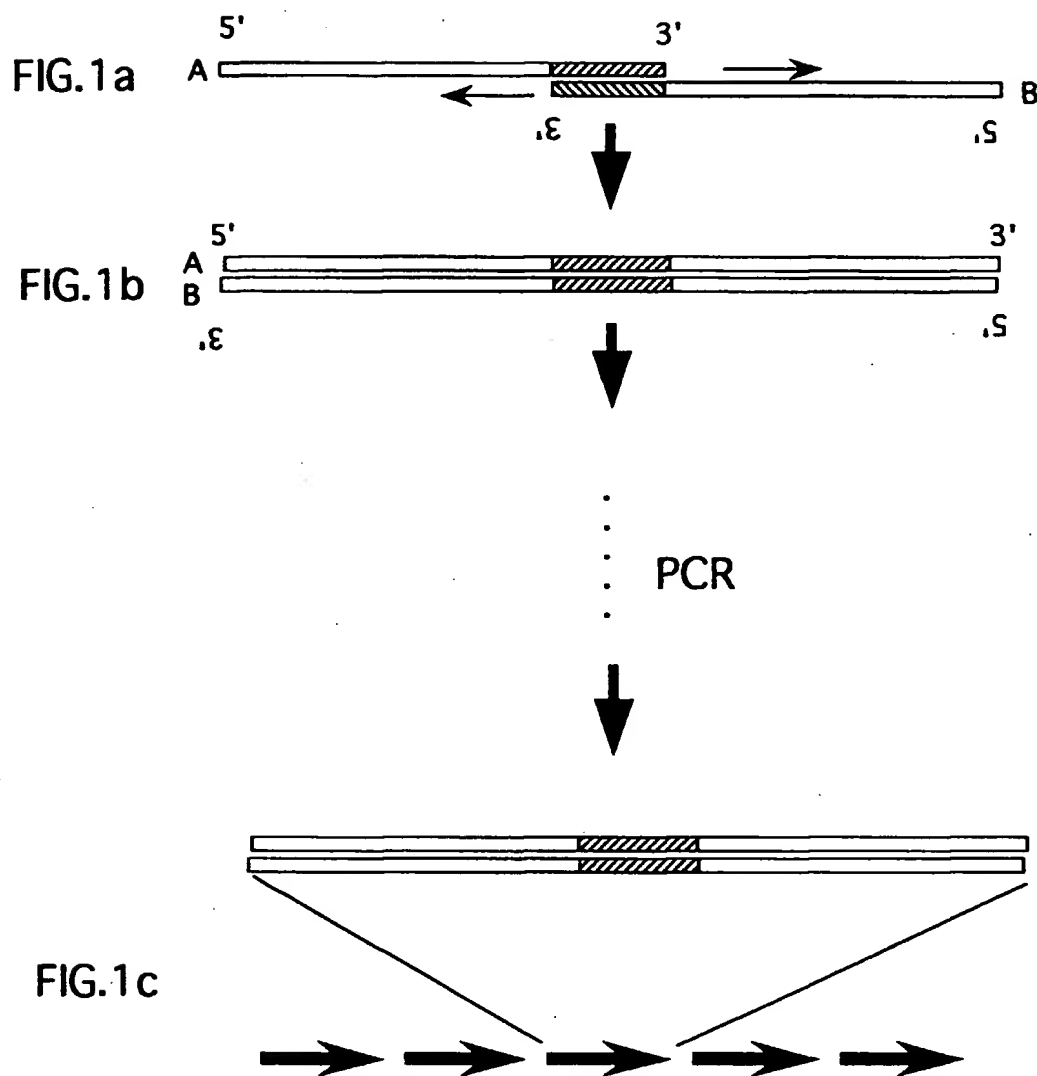


FIG.2

794 GACGGTCACCTGCACAAAGGCG
795 AGTTTCCGCACGTACCTAGGGC



1. Size Marker
2. KY-794 & KY-795

FIG.3

GACGGTCACCTGCACAAAGGCG KY-794
AGTTTCCGCACGTACCTAGGGC KY-795

(pSA32)

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG

(pYT8)

GACGGTCACCTGCACAAAGGCGTGCAGTAGATCCCGCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGC
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCTGCACAAAGGCG

(pSA33)

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGT
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCA
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCGCCA
GACGGTCAC

(pYT5)

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGT
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCCG
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCCCG
GACGGTCACCTGCACAA

FIG.4

794 GACGGTCACCTGCACAAAGGCG
795 AGTTTCCGCACGTACCTAGGGC

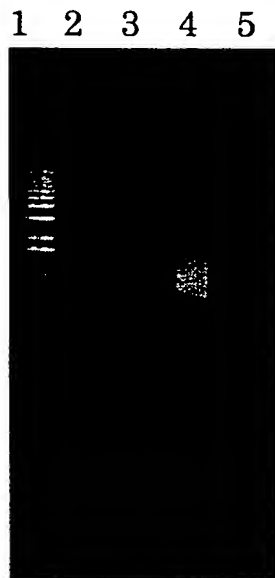
794 GACGGTCACCTGCACAAAGGCG
783 GTTTCCGCACGTACCTAGGGC



1. Size Marker
2. KY-794 & KY-795
3. KY-794 & KY-783

FIG.5

794 GACGGTCACCTGCACAAAGGCG
 795 AGTTTCCGCACGTCACCTAGGGC
 845 GACGGTCACCTGCACAGGCG
 846 AGTCCGCACGTCACCTAGGGC



1. Size Marker
2. KY-794 & KY-795 63°C
3. KY-845 & KY-846 63°C
4. KY-794 & KY-795 66°C
5. KY-845 & KY-846 66°C

FIG.6

794 GACGGTCACCTGCACAAAGGCG
795 AGTTTCCGCACGTACCTAGGGC



1. Size Marker
2. *Pfu* DNA Polymerase
3. Exo⁻ *Pfu* DNA Polymerase

FIG.7

794 GACGGTCACCTGCACAAAGGCG
795 AGTTTCCGCACGTCACCTAGGGC

808 GACGGTCACCTGCAAACGGAGC
809 ATTGCCTCGACGTCACCTAGGGC



1. Size Marker
2. KY-794 & KY-795
3. KY-808 & KY-809

FIG. 8

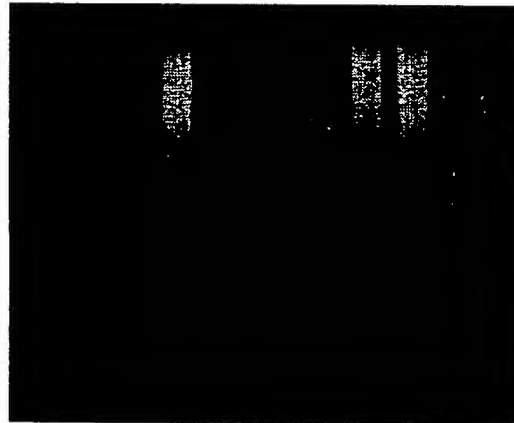
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794  GACGGTCACCTGCACAAAGGCG
827  CTGGGTCACCTGCACAAAGGCG
828  GACCCACACCTGCACAAAGGCG
829  GACGGTGTGCTGCACAAAGGCG
830  GACGGTCACGACCACAAAGGCG

795  AGTTTCCGCACGTCACCTAGGGC
831  AGTTTCCGCTGCTCACCTAGGGC
832  AGTTTCCGCACGAGTCCTAGGGC
833  AGTTTCCGCACGTCAGGAAGGGC
834  AGTTTCCGCACGTCACCTTCCGC
835  AGTTTCCGCACGTCACCTAGGCG

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1 2 3 4 5 6 7 8 9 10 11



1. Size Marker
2. KY-794 & KY-795
3. KY-827 & KY-795
4. KY-828 & KY-795
5. KY-829 & KY-795
6. KY-830 & KY-795
7. KY-794 & KY-831
8. KY-794 & KY-832
9. KY-794 & KY-833
10. KY-794 & KY-834
11. KY-794 & KY-835

FIG.9

GANGGTCACCGGCACAAAGGCG

KY-812

AGTTTCGGCACGTACCTAGGGC

KY-795

(N = A, T, G, C)

(pYT15)

GACGGTCGCGGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GATGGTCACAGCACAAGGCGTGCAGTGGATCCCA
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACAGCACAAGGCGTGCAGTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCAGG

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACAGCACAAGGCGTGCAGTGGATCCCA
 GAAGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCGCGG
 GAAGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GAAGGTCACCGGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACAGCACAAGGCGTGCAGTGGATCCCG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GATGGTCACCGGCAC

(pYT16)

GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACAGCACAAGGCGTGCAGTGGATCCCA
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GATGGTCACCGGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGT
 GAGGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCAC

(pYT22)

GAGGGTCACCGGCACAAAGGCGTGCAGTGGATCCCGCGG
 GACGGTCACCTGCACAAAGGCGTGCATTGGATCCCGCGG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGG
 GACGGTCACCGGCACAAAGGCGTGCAGTGGATCCCA
 GATGGTCACCGGCACAAAGGCGTGCAGTGGATCCCA
 GATGGTCACCGGCACAAAGGCGTGCAGTGGATCCCGCGG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCA
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GAAGGTCACCGGCACAAAGGCGTGCAGTGGATCCCG

(pYT20)

GGTCACCGGCACAAAGGCGTGCAGTGGATCCCGCGG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCG
 GACGGTCACCTGCACAAAGGCGTGCAGTGGATCCCGCGG

FIG.10



1. Molecular Weight Marker
2. pTZ19R/BL21(DE3)
3. pYT32/BL21(DE3)
4. pYT33/BL21(DE3)